ORIGINAL ARTICLE

SCREENING FOR *CHIKUNGUNYA VIRUS* IN HEALTHY BLOOD DONORS: EXPERIENCE FROM BLOOD BANK IN KARACHI PAKISTAN

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ABSTRACT

Background: *Chikungunya virus (CHIKV)*, responsible for a debilitating febrile illness, has seen an increase in prevalence among Pakistani population in recent years. Though mainly transmitted through bite of *Aedes* mosquitoes, the other transmission route of particular concern is transfusion-related, especially during the outbreak season when asymptomatic individuals are capable of transmitting the virus through this route. Therefore, we designed this study to know the infectious and immune status of healthy blood donors against *CHIKV*.

Material and Methods: This was a cross-sectional study conducted on healthy blood donors at the Blood bank of Aga Khan University from 1st July 2018 to 28th February 2019. The sampling method employed was consecutive sampling. Three hundred and sixty healthy blood donors were screened for the presence of *CHIKV* IgM antibodies and nucleic acid using ELISA method and RT-PCR, respectively.

Results: Of the 360 blood donors screened, 1.1% (n=4) tested positive for *CHIKV* IgM however, none were found to be viremic at the time of blood donation. The mean age of seropositive donors was 32.5 years (IQR: 37.5 – 28) and all were residents of Karachi city. *CHIKV* seropositivity was significantly associated with residence in the central district of Karachi (p=0.02).

Conclusion: This screening study is suggestive of potential subclinical infection to *CHIKV* among healthy blood donors and provides evidence for routine screening of this virus to eliminate risks of transfusion related transmission.

Keywords: Transfusion, Transmission, *CHIKV*, Donors

BACKGROUND

Arbovirus infection is predominantly based on transmission cycles that involve interaction between arthropod-borne vectors and susceptible hosts, influenced by various biological and environmental factors.[1] Of the medically important arboviruses, *Chikungunya virus (CHIKV)*, an RNA virus belonging to the alphavirus genus of the family Togaviridae, is responsible for a debilitating febrile disease frequently involving muscles and joints.[2] According to WHO, the emergence and rapid spread of *CHIKV* has occurred in 60 different countries worldwide in the form of sporadic and epidemic outbreaks.[3] Epidemiological analysis suggests different *CHIKV* genotypes to have geographic predominance; hence its categorization into four regional genotypes namely West African, Eastern/Central/South African (ECSA), Asian and Indian Ocean Lineage (IOL). [4]

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Pakistan, the fifth most populous country, owing to its subtropical location and rapid urbanization in the past couple of decades has been at particular risk for *CHIKV* outbreaks. Overcrowding of large cities, underdeveloped housing societies, improper sewage disposal, ineffective environmental cleaning, and inadequate arbovirus control strategies are some of the most important factors responsible for outbreaks in this region.[5] Pakistan has recently faced a sporadic epidemic of *CHIKV* in 2016 and reported up to 8387 suspected and laboratory confirmed cases.[6]

Aedes mosquitoes, the vectors of *CHIKV*, have been found to breed in the mid-town area in close proximity to the Malir and Liyari rivers of Karachi, the southern port city of Sindh province.[7] During monsoon season, heavy rainfalls often lead to over flooding of these rivers resulting in stagnation of water providing potential breeding sites for mosquitoes.[8] In addition to the bite of mosquitoes, the other transmission route of particular concern is transfusion-related, especially during the outbreak season when asymptomatic individuals are capable of transmitting the virus through this route. This mode of transmission of arboviruses has been well documented in case reports

from several countries including Singapore, Honk Kong, Puerto Rico, Brazil, USA and China.[9-14]

There is limited surveillance of arboviruses at a public health level in Pakistan while laboratory diagnosis for arboviruses other than *Dengue virus (DENV)* is virtually non-existent. There are two main sources for case reporting, the first is through diagnostic laboratories that primarily test for and report *DENV* infection to the health authorities and second is syndromic disease surveillance conducted by the Federal Disease Surveillance and Response Unit division of Field Epidemiology & Disease Surveillance at National Institute of Health.[15] Consequently, *DENV* is erroneously considered to be the only endemic arbovirus circulating in Pakistan. However, our group has recently reported evidence of circulation of arboviruses other than *DENV*, including *WNV* and *CHIKV* in the southern region of Pakistan through active disease and vector surveillance conducted during 2015-17.[16, 17]

Though one case of transfusion transmitted *DENV* has been reported in the past from Karachi city[18] the risk that other arboviruses such as *CHIKV* pose to blood safety is unknown. Hence, in the present study we screened healthy blood donors for potential transfusion related transmission of *CHIKV*.

MATERIAL AND METHODS

This study was conducted at the blood bank and the clinical microbiology sections of the Department of Pathology and Laboratory Medicine, Aga Khan University. It was a cross-sectional study conducted on blood donors who presented to the blood bank from 1st July 2018 to 28th February 2019. The sampling method employed was consecutive sampling. Three hundred and sixty healthy blood donors were screened for *CHIKV* IgM antibodies using the commercially available CHIKjj *Detect*™ IgM ELISA kit (Inbios International, Inc USA) and nucleic acid detection by Reverse transcriptase Polymerase chain reaction (RT-PCR).

If the potential donors met the inclusion criteria, then informed and written consent was taken on a consent form after they were explained about the tests that will be conducted on their blood samples. The study was exempted from ethical approval by the Institutional Review Board of Aga Khan University (Ref # 2019-0488-2928).

All the potential donors who presented to the blood bank were initially screened for inclusion in the study, the inclusion criteria was based on eligibility for blood donation. Potential donors equal to or above 18 years of age were included in the study. The individuals who met the following criteria were excluded from the study viz. (i) less than 18 years of age, (ii) presence of fever, (iii) haemoglobin below 12 gm/dl, (iv) currently taking any antibiotic, (v) had a blood transfusion within past 12 months, (vi) donated blood within past 4 months, (vii) had any organ, tissue or bone marrow transplantation within past 12 months, (viii) presence of any chronic disease such as AIDS, cancer, kidney, liver, heart disease etc., (ix) presence of any bleeding condition and (x) presence of any blood borne disease.

Baseline, demographic data and clinical information regarding blood donors was collected on a standardized study proforma. Information regarding donor's age, gender, location, blood group and any history of fever were recorded. The recipients of blood and blood products were followed after transfusion for the occurrence of any transfusion reactions for the duration of their hospital stay.

Donor information, ELISA and PCR results collected on hard copies of the study questionnaires were entered into Microsoft Excel (Microsoft Corporation; version 16.0.12026.20174 / September 17, 2019). Data regarding donor's age, gender and blood group was tabulated in accordance with positive and negative results of serological and PCR testing. The entered data was twice matched for proper verification of transfer from the hard copies to the statistical software.

For the purpose of statistical analysis the data was entered in to Statistical Package for Social Science program (SPSS) (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Statistical significance was analyzed for the association of age, gender, blood group and residence in the Karachi city districts with the serological and PCR results by calculating odds ratio with their confidence intervals. Fisher exact test was used to determine the p value where data values were too small.

RESULTS

Of our blood donor population, 99.2% (n=357) were males and 0.8% (n=3) were females, with a median age of 28 years (IQR: 24–34) (Table-1). Majority of the donors were residents of the urban regions of Sindh province (n=302). *CHIKV* IgM antibodies were detected in 1.1% (n=4) of the donor samples tested whereas, none of the blood donors were found to be viremic at the time of donation and PCR signals remained negative for all samples tested. No transfusion reactions were observed in any of the recipients of blood products from those donors.

All the seropositive donors belonged to Karachi, with majority belonging to the city's central district (75%). This association reached statistical significance [OR= 14.8 (95% CI 1.5-144.7) (p=0.02)]. However, no statistical significance with any of the age or blood groups was noted. Table-2 shows the association of baseline and demographic data with CHIKV ELISA positivity.

Table-1: Demographic characteristics of the donors (n=360) screened for *CHIKV* (July 2018 – Feb 2019).

Characteristic	Total sample n (%)
Gender:	
Male	357 (99.2)
Female	3 (0.8)
Age:	
19-30	217 (60.3)
31-40	108 (30)
41-50	30 (8.3)
>51	5 (1.4)
Residence:	
Rural Sindh region	33 (9.2)
Urban Sindh region	302 (83.9)

Table-2: Association of baseline and demographic data with *CHIKV* ELISA positivity.

Baseline and Demographic Characteristics n (%) OR (95% CI)					
Karachi City Districts					
East	**West**	**Central**	**South**	**Malir**	
0	0	**3 (4.8%)*** **14.8 (1.5-144.7) (p=0.02)**	1 (4.8%) 5.6 (0.6–56.3) (p=0.14)	0	
Age Group					
19-30 y		31-50 **y**		**>50 y**	
2 (0.9%) 0.7 (0.1-4.7) (p=0.68)		2 (1.5%) 1.6 (0.2-11.6) (p=0.63)		0	
Blood group					
A+	**AB+**	**O+**	**O-**	**Rh+**	**Rh-**
1 (1.4%) 1.2 (0.01-11.8) (p=0.87)	1 (4.9%) 5.1 (0.5-50.7) (p=0.12)	1 (0.9%) 0.7 (0.1-6.9) (p= 0.77)	1 (7.8%) 9.6 (0.9-98.7) (p=0.06)	3 (0.9%) 0.3 (0.03-3.2) (p=0.34)	1 (2.8%) 3.1 (0.3-30.2) (p=0.34)

Note: *p value <0.05

DISCUSSION

Data is suggestive of potential subclinical infection to *CHIKV* in 1.1% of healthy blood donors screened in this study.

There has been evidence of circulation of *CHIKV* in Pakistan even before its recognized outbreak in 2016. In a study conducted in Pakistan in 2015, Barr K. and colleagues found 10% positivity for IgM antibodies and 6% for nucleic acid detection in 997 patients with clinical symptoms suggestive of *CHIKV* disease.[16] Subsequently, a massive outbreak of *CHIKV* with approximately 30,000 clinically suspected and more than 4000 laboratory confirmed cases, was reported from Pakistan beginning from November 2016 to April 2017.[6] Despite the increasing prevalence of *CHIKV* infection, surveillance of arboviruses other than *DENV* is limited at the public health level in Pakistan. In addition, blood donor screening for arboviruses has been primarily focused on *DENV* and Malaria. To the best of our knowledge, this is the first study in Pakistan that has sought for screening healthy blood donors for potential *CHIKV* infection using ELISA and nucleic acid amplification tests.

The mean age of seropositive blood donors in this study was 32.5 years, although no statistical significance was noted for any of the age groups. A previous screening study from Pakistan which reported a statistically significant correlation of *CHIKV* positivity in patients with a mean age of 37.6 years, corroborate with our findings.[16] The predominance of *CHIKV* in the young adult and middle aged population might be reflective of the overall age demographics of this region where a significant percentage of people

fall in these age groups.[19] Moreover, this age population is likely to be more social, attending crowded places and working outdoors for longer periods of time. Additionally, laborers and farmers often sleep outdoors at night making them more susceptible for acquisition of mosquito borne infections.

We noticed an overall gender bias in the blood donor population with 99% of subjects comprised of male donors. One of the factors is attributed to the minimum hemoglobin level requirement of 11.5-12.5 g/dL for women. In Pakistan, most women suffer from mild anemia.[20] Therefore, there is little inclination and trend to donate blood among the female population. Hence, all the donors who screened positive for *CHIKV* IgM were males in our study.

In the past decade, there has been a rapid rise in arbovirus related infections including *chikungunya* in mega cities around the world. This is primarily due to overcrowded housing societies with improper waste disposal and mosquito breeding control environment.[5] Karachi, the premier metropolitan city of Pakistan has been devastated with heavy rainfalls in the past experiencing several arbovirus related outbreaks.[8] All blood donors who screened positive were residents from Karachi with majority from the city's central district. This association reached statistical significance (p=0.02) and is likely due to the close proximity of the central city district to the Liyari river in which the district's waste is disposed. This river has been reported to be the breeding site of *Aedes* mosquitoes in a past vector surveillance study.[7]

At the time of screening, all seropositive blood donors were asymptomatic. Other blood donor screening studies conducted in the past correlate with this finding. A cross-sectional study from Dares Salaam reported 2.97% positivity for *CHIKV* IgM in asymptomatic blood donors.[21] Similarly, a study conducted in Myanmar showed 2.8% *CHIKV* IgM reactivity among blood donors who lacked any symptoms of *CHIKV* disease.[22]

All IgM positive blood donors were negative for *CHIKV* RNA on RT-PCR. There have been blood donor screening studies in the past that have reported the detection of *CHIKV* IgM antibodies in the absence of viremia.[22] After the onset of symptoms, *CHIKV* detection in serum can take up to 7 days. IgM antibodies against *CHIKV* usually start appearing at 4th day after onset of symptoms and peaking at day 7. They can remain in the serum for up to 3 months. Therefore, sero-reactivity to IgM antibodies in the absence of viremia may indicate either subclinical or recent infection.[23] Hence, molecular testing may provide a useful aid in addition to serology in preventing transfusion related transmission of *CHIKV*.[24] As we performed real time PCR on donor samples, which has been demonstrated in some studies to have a relatively lower sensitivity as compared to nested PCR, might be the reason for the negative PCR results in donor population.[25]

There were a few limitations to this study. Firstly, this was a single center study. However, the blood bank of Aga Khan University Hospital is one of the largest in Pakistan and donors belong to all over the country. One of the limitations of this study was that we used conventional RT-PCR method for detecting viremia, although we have used this PCR protocol successfully in detecting viral genome in symptomatic patients, however, its sensitivity for the screening purposes in the donor population may have been bit compromised due to low viremia. Further studies using multiplexed nested PCR, are recommended with active clinical follow up of the recipients of the blood products from *CHIKV* IgM positive donors for any transfusion related infections after they were discharged was not performed, however as per blood bank protocol recipients were instructed to contact in case of any possible transfusion linked symptoms. We recommend further studies with proper recipient follow up and more sensitive nucleic acid detection methods to truly monitor the risk of transfusion related transmission of *chikungunya* and other arboviruses infections.

CONCLUSION

To the best of our knowledge this is the first report of *CHIKV* screening in blood donors from Pakistan. We conclude that the risk posed by *CHIKV* to blood transfusion safety exists due to the active circulation of the virus in our population. Prospective multi-center studies with more robust follow up and virus detection methods are required to assess the burden of transfusion associated transmission of arboviruses providing evidence for regular screening of blood donors.

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